Methods: Chondrocytes, MSC and a mixture of chondrocytes and MSC (1:1) were embedded in a 3D-fibringel and either kept in monoculture or were cocultured with cartilage or subchondral bone explants (obtained from arthroplastic surgery due to OA) for up to 28 days. Matrix production and chondrogenic characteristics of cells were determined immunohistologically (collagens I, II and III) and histologically (aggrecan) after 7, 14, 21 and 28 days. Gene expression of *SOX9, COL1A1, COL2A1, COL3A1* and *COL10A1* and of two miRNAs was analyzed after 7 and 28 days with quantitative real-time PCR. Protein expression in the cell-fibrin constructs was assessed with ELISA (collagen I / II), western blot (Sox9, phospho-Sox9 and collagen III) and glycosaminoglycan (GAGs) production was analyzed using a DMMB-assay. The culture supernatant was analyzed for collagen fragments by a hydroxyproline assay and for PTHrP with an ELISA. We separated the supernatants from MSC - monocultures versus cartilage cocultures with SDS-PAGE and identified differentially secreted proteins with MALDI-TOF.
Results: MSC underwent chondrogenic differentiation with respect to collagen II expression in all culture conditions. In comparison to monocultures, collagen I, II and III gene expression and protein production was suppressed when MSC or chondrocytes were cocultured with cartilage or subchondral bone explants. Mixed cultures of chondrocytes and MSC showed a reduced collagen II production in all culture regimes. GAG production and total collagen content in the supernatant was altered in bone explant cocultures only. Phospho-Sox9 and PTHrP protein expression were not significantly affected by any culture condition. However, preliminary data indicate a correlation between miRNA expression and regulation of sox9 protein. MALDI-TOF analysis revealed several proteins/factors present in coculture supernatants but not in the monocultured controls (e.g. stromelysin-1, chondroadherin, fibronectin and SPARC).
Conclusions: Chondrogenic differentiation of MSC is affected by the microenvironmental conditions. Cartilage and subchondral bone explants from OA-patients suppress collagen I, II and III gene expression and production in MSC. This observation might be partly dependent on regulation of sox9 protein biosynthesis by miRNA. Future studies with ovine tissue will delineate if healthy cartilage/subchondral bone provokes different responses in MSC compared to OA derived tissues.
Chondroadherin, fibronectin, SPARC and stromelysin-1 among other, yet unidentified proteins, were detected in coculture supernatants and might be associated with the reduced collagen content in OA-cartilage/subchondral bone cocultures of MSC. We will study a putative link between these factors and regulation of collagen expression and degradation in further analyses.

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T3 EFFECT THROUGH SCR2 ON CHONDROGENESIS IN VITRO

M.C. Arufe [1,2], A. De la Fuente [2], P. Fernández-Pernas [1], I. Fuentes-Boquete [2], F. De Toro [2], F.J. Blanco [1,2]. [1] *Dept. of Med.. Faculty of Hlth.Sci.. INIBIC-CHUAC, A Coruña, Spain;* [2] *Osteoarticular and Aging Res. Lab.. INIBIC-CHUAC, A Coruña, Spain*

Purpose: There are evidences about the thyroid hormone role into chondrogenesis but its molecular implication is not clear. The current study treats to answer this question working about a new *in vitro* model of chondrogenesis using mesenchymal stem cells (MSCs) from human umbilical cord stroma tissues
Methods: The umbilical cord tissues were obtained from caesareans from healthy women in the Maternity Facility at Complejo Hospitalario Universitario de A Coruña under the supervision of the hospital ethic committee. MSCs were isolated from umbilical cord stroma tissue using an enzymatic digestion and cultured the cells adhered to the plastic plate. Chondrogenic process was performed using our model which consisted in growing the cells during two days in medium with FCS 10% in DMEM. After 2 days the medium was replaced by a medium with KO serum and TGF-ß3 which induces the chondrogenesis. Different amounts of human T3 (1, 10 or 100 ng/mL) were adding to the medium to test their role into chondrogenesis process, also different experiments with T3 alone or together with M151, a specific steroid covalent receptor type 2 (SCR2) inhibitor, in the chondrogenic medium, were performance to check which was the T3 receptor which had a active role in the chondrogenesis. Spheroids made by this method were collected after 7, 14 and 28 days in culture and storage at -20ºC for their posterior analysis. Immunohistochemistry analysis of spheroids against COL2, COL1 and aggrecane stain was performed to check chondrogenic differentiation stage and quantitative RT-PCR to check expression of COL2, COL1 and SOX9 genes also was made. Furthermore, proteins involved into Wnt (Beta-catenin and GSK3-B) and Notch (Notch2 and Jagged) pathways were analyzed by western.
Results: Immunohistochemistry analysis of the tissues from the spheroids demonstrated that 100 ng/mL of T3 improved significantly ($P<0.05$) the COL2 and aggrecane staining after 14 days into chondrogenic medium versus chondrogenic medium without T3. This improving was totally withdrawal when the SCR2 inhibitor, M151, was added to the medium at 2.5 ng/mL dose. Quantitative RT-PCR analysis confirm the immunohistochemistry results, improving significantly ($P<0.05$) the COL2 and aggrecane gene expression after 14 days into chondrogenic medium when 100 ng/mL of T3 was present and revealed that expression of COL1 was significantly increased ($P<0.05$) when M151 (2.5 ng/mL) was added to this medium. Western analysis indicated that Beta-catenin was increased in the spheroids when T3 (100 ng/mL) was in the medium after 14 days (2 fold) with respect to medium alone and was decreased until basal levels found in spheroids growth into medium without T3, when M151 (2.5 ng/mL) was present in the medium. Notch2 and Jagged was decreased (2 fold) during the chondrogenesis when T3 was present in the medium with respect to medium alone and the opposite effect was found when M151 was present in the medium.
Conclusions: T3 is improving the chondrogenic differentiation of mesenchymal stem cells from umbilical cord tissue. That effect was made through SCR2 as well as T3 is associated the expression of B-catenin and Notch2 in our chondrogenic model.

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INTRA-ARTICULAR INJECTION OF ADIPOSE-DERIVED STEM CELLS PREVENTS CARTILAGE DESTRUCTION, NEW BONE FORMATION IN THE LIGAMENTS AND LOWERS SYNOVIAL ACTIVATION

M.C. ter Huurne [1], P.L. van Lent [1], A.B. Blom [1], R. Blattes [2], L. Casteilla [2], C. Jorgensen [3], W.B. van den Berg [1]. [1] *Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands;* [2] *STROMALab UPS/CNRS UMR5273, EFS, INSERM U1031, Toulouse, France;* [3] *Inserm, Montpellier, France*

Purpose: Synovial activation is evident in a substantial subpopulation of patients with early osteoarthritis (OA) and has been associated with pathophysiology and clinical symptoms of OA. Previous studies have shown that activated synovial macrophages are involved in mediating cartilage destruction as well as the formation of osteophytes during experimental OA. Recently it has been shown that Adipose-derived Stroma/Stem Cells (ASCs) express immunosuppressive characteristics. The aim of our study was to explore the effect of an intra-articular injection of ASCs on joint pathology during experimental OA.
Methods: ASCs were isolated from inguinal fat surrounding the popliteal lymph nodes and cultured for two weeks according to standard procedures. ASC were characterized with FACS analysis on a set of specific cell surface markers. Experimental OA was induced by injection of collagenase into murine knee joints, which causes instability and cartilage destruction. Collagenase-induced OA is characterized by thickening and activation of the synovial lining layer. ASCs were injected into knee joints at various time-points after induction of OA. OA phenotypes were measured within 8 weeks after induction. Total knee joints were isolated and processed for histology. Synovial activation was measured using an arbitrary scale (0 to 3) and cartilage destruction was measured in 4 different layers of the knee joint (medial and lateral tibia and femur) according to the scoring method of Pritzker et al. 2006. Moreover, newly formed bone in the ligaments was quantified using image analyses.
Results: After culture FACS analysis showed that the adherent fraction expressed characteristic markers for stem cells (positive staining for Sca-1, CD-44 and CD-105 and negative staining for CD-11b and cKIT). A single dose of ASCs ($20x10^3$ in mouse serum) was injected into the knee joint of mice, 7 days after induction of osteoarthritis. Synovial activation was inhibited at day 14 (9%) and day 42 (31%) ($P< 0.05$) after treatment when compared to serum injected knee joints. Destruction of cartilage was also inhibited at day 14 and significantly at day 42 (35%). Inhibition of cartilage

destruction was particularly found at the lateral plateau (48% lower). Interestingly, ASC-treatment had a protective effect on new cartilage and bone formation in the ligaments. At day 42, the surface percentage of the cruciate ligament which stained positive for proteoglycan deposition was decreased by nearly 43% in the ASC treated joints when compared to controls. In line with that, osteophyte formation in the medial collateral ligament was significantly reduced by 91% in ASC-treated animals compared to control. In contrast to early treatment, injection of the same dose of ASCs, 14 days after induction of OA only showed a small inhibiting effect on osteophyt formation and synovial activation when measured at day 42. Although cartilage destruction diminished with 28%, these values did not reach significance at that time-point.

Conclusions: Our study indicates that a single injection of ASCs into the knee joints of mice with collagenase-induced osteoarthritis prevents cartilage damage and the formation of bone structures within the ligaments, probably by inhibiting activation of the synovial macrophages.

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CHONDROGENIC AND IMMUNOPHENOTYPIC PROPERTIES OF MESENCHYMAL STEM CELLS FROM OSTEOARTHRITIS PATIENTS

S. Hagmann, B. Moradi, S. Frank, A.-M. Bäsig, T. Dreher, W. Richter, T. Gotterbarm. *Dept. of Orthopedics and Traumatology, Heidelberg, Germany*

Purpose: Mesenchymal stem cells (MSCs) are a promising target for future cell-based therapies in osteoarthritis. In this context, cartilage tissue engineering is a major field of research, with effective cartilage repair being the ultimate objective. There is a controversy whether osteoarthritis and age affect the regenerative potential of MSCs. Also, in the last decade, it has become clear that MSCs are heterogeneous populations not only regarding their regenerative potential, but also regarding their surface markers. The aim of this study was to analyse the influence of osteoarthritis and age on growth parameters, immunophenotype and chondrogenic differentiation potential of human bone marrow derived MSCs.

Methods: MSCs were isolated from bone marrow of osteoarthritis patients (n=5, OA) and patients without clinical and radiological signs of osteoarthritis (n=5, non-OA) and expanded in different cell culture media. Flow cytometry analysis was performed for CD10, CD13, CD14, CD34, CD44, CD45, CD49, CD73, CD90, CD105, CD117, CD133, CD 140b, CD 166, CD271, CD340, Stro-1 and HLA-ABC. Chondrogenic differentiation was induced with chondrogenic medium in pellet culture. Chondrogenic differentiation potential was evaluated by a DMMB-assay and by semiquantitative analysis of GAG percentage (SafraninO/FastGreen staining). Statistical analysis as performed with analyses of variance (ANOVA).

Results: Both in the OA and the non-OA group, MSCs were negative for CD14, CD34 and CD45 and positive for CD13, CD44, CD73, CD90, CD105 and CD166. CD49, CD105, CD140b, CD146, CD340 and Stro-1 showed differences depending from the media applied, but no significant differences were observed between the OA and non-OA group. The expression of the MSC surface markers CD10, CD49 and CD146 showed high donor dependence while for these markers there was no significant difference between the OA and the non-OA group. Cell proliferation differed substantially between different culture media while the growth index parameters were similar in the OA and non-OA group. There was no difference in MSC chondrogenic potential between OA and non OA patients while the culture media had an important influence on chondrogenic differentiation.

Conclusions: Our findings add to the reports that negate the regenerative inferiority of MSCs from OA affected patients. Our experiments rather suggest that MSCs extracted from OA patients are an adequate source for cartilage tissue engineering, which is supported by the finding that MSC surface markers do not seem to be influenced by age or presence of OA. Our data also reflects that MSCs are heterogeneous regarding some of their surface markers and that culture conditions seem to have an important influence on both immunophenotype and chondrogenic potential. In the long-term, understanding the potential of MSC heterogeneity and of culture conditions will hopefully lead to more efficient cartilage tissue engineering.

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LONG-TERM EFFICACY OF MESENCHYMAL STEM CELLS IMPLANTATION IN COMBINATION WITH TYPE I COLLAGEN MEMBRANE: A RAT EXPERIMENTAL MODEL OF ROTATOR CUFF TEARS.

P. Tornero-Esteban, C. Rodriguez-Bobada, J. Hoyas, E. Villafuertes, F. Marco, B. Fernandez-Gutierrez. *Hosp. Clinico San Carlos, Madrid, Spain*

Purpose: The Supraspinatus tendon (SE) is an essential structure for the proper function of the rotator cuff frequently affected in rotator cuff tears. Common surgical procedures to repair SE lesions are usually adequate in the short term, but they are often unsatisfactory in the long term presenting a high rate of re-rupture. In this context, there is a need for new techniques allowing a more efficient repair. Regenerative medicine using mesenchymal stem cells (MSCs) offers a promising alternative. This study explore in a rat model the possible benefits of a treatment based on MSC-collagen membrane graft compared to conventional treatment (suture) or collagen membrane graft.

Methods: A chronic rotator cuff tear injury model was developed by unilaterally detaching the SE tendon of adult Sprague-Dawley rats. One month post-injury, the tendon was repaired by: a) Classical surgery, using single suture. b) suture + type I collagen membranes without cells; Membrane or c) suture + type I collagen membranes + $1x10^6$ allogeneic MSCs; (n=12; MSC-Membrane). Lesion was evaluated at 1 month, 2 months and three months post-injury by biomechanical criteria including "load-to-failure" measured (Newtons), Stifness (N/mm) and deformation (mm). The opposing and not injured shoulder was used as positive control after normalization. Non-parametric Mann-Whitney U test was used for statistical analysis, $p < 0.05$ was considered significant.

Results: Biomechanical properties (median [25%-75% percentil]) of tendon repair

Conclusions: We observed that implanting MSC-Membrane into surgically created tendon defects improved the strength and stiffness of the repaired tendon at three months, indicating that the reparation process ameliorated after long-term. This likely indicates that long-term efficacy is influenced by underlying mechanism improving the natural tendon repair.

	Months	n	Suture	n	Membrane	n	MSC-Membrane
Load to failure (N)	1	4	1.31 [1.19-1.69]	6	0.73 [0.35-1.23]	6	1.04 [0.77-1.22][b]
	2	4	1.22 [0.76-1.60]	4	0.99 [0.63-1.59]	5	1.11 [0.74-1.39]
	3	3	1.14 [0.28-1.29]	5	0.94 [0.84-1.27]	4	1.70 [1.27-1.79][a,b,c]
Total n		11		15		15	
Stiffness (N/mm)	1	4	1.20 [0.99-1.42]	6	0.62 [0.44-0.88]	6	0.63 [0.46-0.90]
	2	4	0.86 [0.49-0.91][c]	4	0.87 [0.53-2.15]	5	0.66 [0.47-1.05]
	3	3	0.70 [0.37-0.90][a]	5	0.91 [0.60-2.03]	4	1.16 [1.04-1.32] [a,b]
Total n		11		15		15	
Deformation (mm)	1	4	1.13 [0.77-1.40]	6	1.12 [0.84-1.66]	6	1.21 [0.83-2.02]
	2	4	1.53 [1.13-1.75]	4	1.01 [0.82-1.65]	5	0.98 [0.84-1.92]
	3	3	1.15 [0.68-1.68]	5	1.32 [0.54-2.30]	4	1.30 [0.84-1.42]
Total n		11		15		15	

[a] Statistically significant from same group repair.
[b] Statistically significant from Suture *vs* MSC-Membrane
[c] Statistically significant from Membrane *vs* MSC-Membrane